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Simultaneous absorbance-ratiometric, fluorimetric, and colorimetric analysis and biological imaging of α-ketoglutaric acid based on a special sensing mechanism

Qiang Fu [a], Zhi Chen [a], Xueying Ma [b], Guang Chen [b,c,*], Yuxia Liu [b], Ziping Cao [b], Fei Qu [a], Daoshan Yang [a], Xianen Zhao [a], Zhiwei Sun [a], Guoliang Li [a], Shijuan Zhang [a], Fengli Qu [a], Rongmei Kong [a], Hua Wang [b,*], Jinmao You [a,b,*]

[a] Qinghai Normal University, Xining, China
[b] The Key Laboratory of Life-Organic Analysis, Key Laboratory of Pharmaceutical Intermediates and Analysis of Natural Medicine, Qufu Normal University, Qufu 273165, Shandong, China
[c] Key Laboratory of Detection Technology for Tumor Markers, College of Chemistry and Chemical Engineering, Linyi University, Linyi 276005, China

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ABSTRACT

Probe for α-ketoglutaric acid (α-KA) usually provides low sensitivity, invisible colour change and complex procedure with surfactants, which limits the accurate, sensitive quantification and mornitoring of α-KA in biological samples. Essentially different from the routine sensing mechanism, a special mechanism of photo-induced electron transfer (PET) combining with large absorption red-shift (rather than emission) has been designed to develop a facile yet multifunctional probe for the simultaneous fluorimetric, absorption-ratiometric and colorimetric detections of α-KA in biological samples. Effective inhibition of PET ensured the intense fluorescence turn-on (60 fold) and qutie low detection limit (0.9 μM) for α-KA without need of any surfactants. Dramatical colour change caused by the large absorption red shift (100 nm) from UV–vis region enabled the colormetric analysis of α-KA by naked-eye. Meanwhile, the large absorption red shift also contributed to the ratiometric detection, allowing for the accurate quantification of α-KA in complex biological sample. With this probe, ratiometric detection, naked eye mornitoring and imaging of α-KA in serum, living cells and tissues are realized for the first time.

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1. Introduction

Biomarker can provide the specific indication for occurrence, progression, as well as diagnosis of different types of diseases [1–5]. For example, α- ketoglutaric acid (α-KA) is produced in tricarboxylic acid cycle catalyzed by isocitrate dehydrogenase (IDH), thereby reflecting the somatic mutation of IDH found in cancers [6,7]. More recently, α-KA has been demonstrated to be a novel biomarker for non-alcoholic fatty liver and breast cancers [8–10]. Therefore, the accurate, sensitive, and convenient measurement of α-KA in vitro and/or in vivo is of great significance for the diagnosis and research of these serious diseases.

Up to date, analytical methods established for α-KA are mainly confined to some sophisticated equipments like gas chromatography (GC), high performance liquid chromatography (HPLC), mass spectrometer (MS) [11], proton magnetic resonance spectrometer [12,13], and ultraviolet spectrometer [14]. Despite the extensive applications, these methods are not suitable for the convenient and instant analysis of biological sample due to the complicated and tedious procedures. In contrast, optical sensing technique has shown its noteworthy advantages in terms of selectivity, sensitivity, analysis time, solvent consumption, and convenience [15]. By virtue of these advantages, optical probes have been widely applied to the non-invasive and real-time detection of biomarkers in biological samples [16–22]. However, optical probe for α-KA detection remains rather scarcely developed. Recently, pioneering works have been reported for the high-throughput monitoring of α-KA with the aid of fluorescent probes [23,24]. Unfortunately, the optical mechanism of those probes led to the unsatisfactory sensitivity unless surfactant was added. More seriously, the complex matrix in biological samples, even to which the complex surfactants

* Corresponding authors at: The Key Laboratory of Life-Organic Analysis, Key Laboratory of Pharmaceutical Intermediates and Analysis of Natural Medicine, Qufu Normal University, Qufu 273165, Shandong, China.
E-mail addresses: chenandguang@163.com (G. Chen), huawang_qfnu@126.com (H. Wang), jmyou6304@163.com (J. You).

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had to be added, might influence the analysis accuracy. Moreover, the reported blue shift in absorption from UV–vis region brought about no visible colour change, and thereby was unsuitable for the naked eye monitoring of α-KA in biological samples. In addition, no imaging of α-KA in cells or tissues is available up to now, probably due to the relatively low sensitivity of the reported probes toward α-KA. These drawbacks limited the study of α-KA in biological sample, to a large extent. Therefore, it is highly desirable to develop a probe having the sensitive response, accurate self-calibration [25], visible colour change, as well as imaging ability for α-KA in various biological samples [26].

In present work, a multifunctional probe featuring a special optical mechanism has been developed for the simultaneous fluorimetric, absorption-ratiometric, and colorimetric detection and biological imaging of α-ketoglutaric acid. Herein, 4-nitrobenzo-2-oxa-1, 3-diazole (NBD) is selected as a fluorophore, owing to its long-wavelength emission, high quantum yield, good cell permeability, low toxicity as well as facile availability [27]. Based on a rational design of photo-induced electron transfer (PET), a simple yet multifunctional probe 4-hydrazinyl-7-nitrobenzo[c][1,2,5]oxadiazole (HNBD) is synthesized. HNBD is designed with the controllable frontier orbitals, by means of which the remarkable off/on switch of fluorescence and the large absorption red shift in response to α-KA was enabled. Correspondingly, sensitive response (60 fold), remarkable colour change (colourless to orange-red) and ratiometric quantification for α-KA are achieved, demonstrating the developed probe a sensitive, accurate and convenient alternative to previous α-KA probes. In addition, this multifunctional probe is successfully used for the detection as well as imaging of α-KA in serum, living cell, and tissue. To the best of our knowledge, this is the first attempt for ratiometric quantification, colorimetric analysis and biological imaging of α-KA.

2. Experimental

2.1. Reagents and instruments

All solvents used were of analytical grade without further purification. HPLC–MS/MS analysis was performed on an Agilent 1290 series HPLC system coupled by an Agilent 6460 Triple Quadrupole MS/MS system that was equipped with an Agilent Jet Stream electrospray ionization source (ESI source). HPLC separation was achieved using a SB C18 column (2.1 mm × 50 mm, 1.8 μm i.d., Agilent, USA). HPLC-UV analysis was carried out on Agilent 1260 series, with an online vacuum degasser, a quaternary pump, an autosampler, a thermostated column compartment and a fluorescence detector equipped. The elution gradient was set as: 0 min: 70% A + 30% B; 10 min: 0% A + 100% B; 15 min: 0% A + 100% B; where A and B were the 0.5% FA + 5% ACN and the 100% ACN, respectively. UV–vis spectra were recorded on Cary 300 Bio UV–vis spectrophotometer. Fluorescence measurements were performed on Hitachi F-7000 fluorescence spectrophotometer, with the excitation (475 nm) and emission (540 nm) slit widths at 10.0 and 10.0 nm respectively. Imaging of cell and tissue were performed with an Olympus, IX73-DP80 (Japan) inverted microscope. TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 300–400), both of which were obtained from the company JianYou (Yan Tai, China). NMR was measured on Bruker Ascend 500 (500.1 MHz, 1H; 125.8 MHz, 13C) instrument operating at the denoted spectrometer frequency given in mega Hertz (MHz) for the specified nucleus. Chemical shifts are given in parts per million (ppm) relative to tetramethylsilane (TMS) as an external standard for 1H- and 13C NMR spectra and calibrated against the solvent residual peak.

2.2. Synthesis of the probe HNBD

To the 50 mL solution of chloroform containing 0.3 g of NBD-Cl (7-chloro-4-nitrobenzo-2-oxa-1, 3-diazole), solution of hydrazine hydrate (0.3 mL) in methanol (50 mL) was added. The resulting mixture was stirred under ambient temperature for 30 min. Then the brown precipitate was washed by ethyl acetate to get the probe HNBD (0.29 g, yields: 97%). ^{1}H NMR (500 M, d_6-DMSO): δ 8.09 (br, 1H), 6.99 (d, J = 10.5 Hz, 1H), 5.83 (d, J = 10.1 Hz, 1H). Note: the peak of amino H (3.39 (d, 2H)) was overlapped by the residual water in solvent. ^{13}C NMR (125.8 MHz, d_6-DMSO): δ 147.99, 145.21, 129.10, 121.38, 113.39, 79.65. HRMS (ESI) calcd. For $C_6H_5N_5O_3$ $(M+H)^+$ m/z 195.04, found: 196.00 (ESI). The probe HNBD was employed to explore α-KA according to the reaction scheme (Fig. 1) and the product HNBD-α-KA was characterized using HPLC-UV (Fig. S1, ESI) and HPLC–MS (Fig. S2, ESI).

2.3. Preparation of test solution

The stock solutions of probe HNBD (2×10^{-4} M) and α-KA (1×10^{-3} M) were respectively prepared with ACN as the solvent. Solutions for selectivity experiments were prepared with various compounds, including amino acids (Alanine (Ala), Cysteine (Cys). Histidine (His), Methionine (Met), Arginine (Arg), Glutamine (Gln), Isoleucine (Ile), Phenylalanine (Phe), Asparagine (Asn), Leucine (Leu), Proline (Pro), Aspartic acid (Asp), Glycine (Gly), Lysine (Lys), Sarcosine (Sar), Serine (Ser), Threonine (Thr), Tryptophan (Trp), Valine (Val), Glutamine (Glu)), Glucose (Glu), Galactose (Gal), Glyoxal (GO), hydrogen peroxide (H_2O_2), sodium pyruvate (PAS), phenylglyoxal (PGO), methylglyoxal (MGO), and phenylpyruvic acid (PPA). The blank solution was prepared by mixing 100 μL of stock solution of HNBD, 50 μL of PBS (10 mM, aqueous, pH 7.4) and 850 μL of ACN. The test solution was prepared by mixing 200 μL of α-KA stock solution, 100 μL of probe stock solution, 50 μL of 10 mM PBS (aqueous) and 650 μL of ACN in 1 mL solution (pH 7.4). All the solutions were shaken well and allowed to stand at 37 °C for 60 min before analysis.

2.4. Fluorescence imaging of cells and tissues

Yeast cells were seeded in yeast peptone dextrose agar (YPD) (Aladdin) for 24 h. Then, yeast cells were incubated with probe HNBD (20 μM) in the culture medium for 12 h at 37 °C. After being washed with PBS three times to remove the remaining probe, yeast cells were incubated with α-KA (200 μM) for another 12 h at 37 °C. The cells were washed by PBS three times to remove the remaining α-KA, and then used for fluorescence imaging. Sample of lean pork tissue was cut to the slice of 1 mm and then incubated with probe HNBD (20 μM) for 1 h at 37 °C. After being washed with PBS three times to remove the remaining HNBD, slices were further incubated with α-KA (200 μM) for 1 h at 37 °C. Then, the slices were washed with PBS three times to remove the remaining α-KA, and the fluorescence images were obtained with the fluorescence microscope.

2.5. Detection of α-KA in serum

Human serum (5 mL) spiked by 1 mL ACN was centrifuged (5000 rpm) for 20 min. Then 4 mL of supernatant was transferred into a tube for test. Four batches of serum samples were prepared: 1: 200 μL of serum (blank); 2: blank + HNBD probe (20 μM), 3: blank + α-KA (200 μM), 4: blank + HNBD probe (20 μM) + α-KA (200 μM), which were then kept at 37 °C for 60 min. Then, linear relationship was established by the responses versus the concentrations of α-KA (0–900 μM).

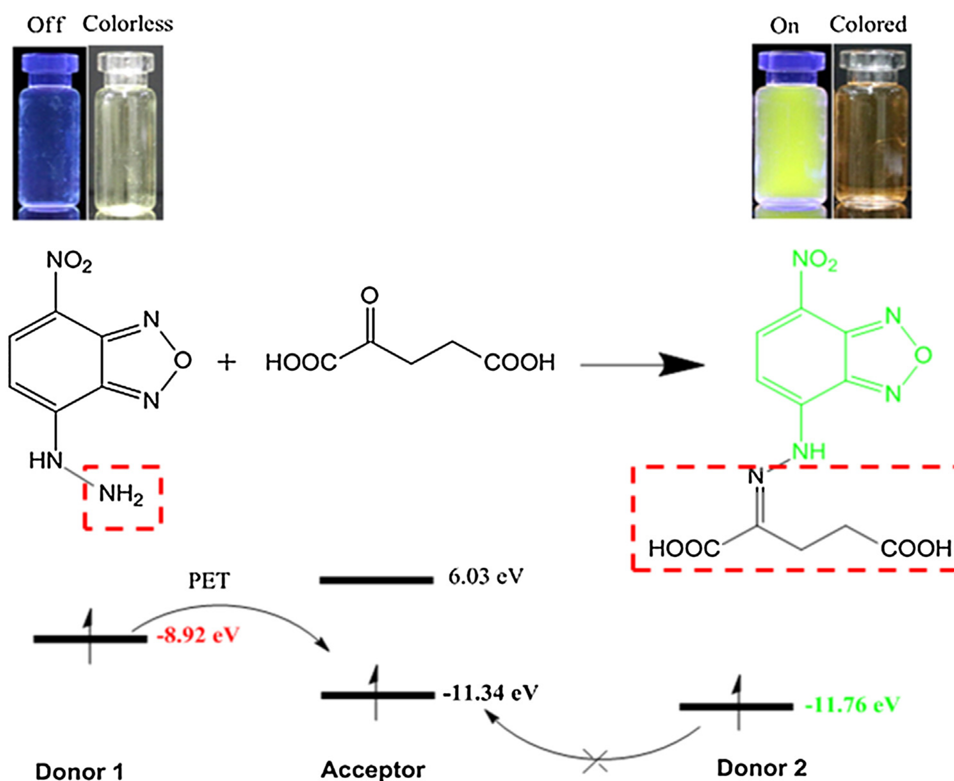

Fig. 1. Schematic illustration of the proposed procedure and the special PET mechanism for the optically "turn-on" reaction of HNBD probe with α-KA, on the basis of density functional theory (DFT) at B3LYP level with the standard 6–31 g (d,p) basis set (Donor 1: amino group; Acceptor: Fluorophore of NBD; Donor 2: α-KA group).

2.6. Computational details

The geometry optimizations of the excited states were performed in the framework of density functional theory (DFT) at B3LYP level with the standard 6-31g(d,p) basis set, which are based on the geometries of the ground states at the same DFT level. To obtain more reliable results, the single-point energy calculations of the excited states were carried out using B3LYP level with the larger 6–311 + g(d,p) basis set for all atoms. All the energies were obtained with Gaussian 09 program.

2.7. Further confirmation of sensing mechanism

In addition to the calculated energy data, a supplementary confirmation of the PET mechanism by fluorescence was performed. Considering the electrons of amino group might be transferred via the PET mechanism, an experiment to inhibit PET by neutralizing the amino group with hydrogen ion was designed. To the solutions of probe HNBD (20 μM) in PBS (0.5 mM, pH 7.4, 95% ACN), various levels of hydrochloric acid (HCl) were added, respectively. The influence of pH on fluorescence intensity of HNBD probe was investigated. Furthermore, to demonstrate the proposed PET mechanism from the perspective of molecular structure, experiments of ^{1}HNMR titration were designed by adding respectively 0. 2, 5, 10 folds molar amounts α-KA to probe HNBD in DMSO-d_6 with micropippet. Spectral data was recorded after a placement of the mixture at 37 °C for 60 min.

3. Results and discussion

3.1. Fabrication and mechanism of probe HNBD

We had been considering that the hydrazino at 7-position of NBD fluorophore might quench the fluorescence via the photo-induced electron transfer (PET) mechanism. When this hydrazino was bonded to α-KA to form a Schiff-base, inhibition of PET would bring about the remarkable turn-on of fluorescence. Meanwhile, we reasoned that the routine blue shift in absorption from about 380 nm (NBD fluorophore) would not result in the colour change observable by naked eye. However, we might modify the NBD fluorophore to reduce the energy gap between frontier orbitals, hoping to achieve the large red-shift of absorption. Thus, the visible colour change, the convenient colorimetric analysis or even the accurate ratiometric detection might be expectable, which was probably the first attempt for α-KA detection, to the best of our knowledge.

With these in mind, we facilely prepared the probe HNBD and then investigated its optical properties in the solution of phosphate-buffered saline (PBS, pH 7.4). As can be seen from Fig. 2a, the emission spectrum of HNBD exhibited a rather weak intensity, indicating that the PET mechanism effectively quenched the fluorescence. To confirm this mechanism, we first investigated the reaction between HNBD with the analyte α-KA by performing an experiment of 1H NMR titration. Fig. S13 showed that, when 2 fold of α-KA was added to HNBD solution in DMSO-d_6, the peaks of $-NH_2$ at 3.39 (Fig. S12) disappeared and the peaks of $-CH_2$ at the chemical shift of 2.80/2.73 (for α-KA) and 2.40/2.29 (for HNBD-α-KA) were observed. As the α-KA increased, peaks for both α-KA and HNBD-α-KA were observed to increase. These results let us infer that probe HNBD will react with carbonyl group in α-KA to form the Schiff base products HNBD-α-KA. Then, based on this product, we estimated the process of PET with the energy level calculated by the density functional theory (DFT) at B3LYP 6-31g(d,p) basis set (ESI) [28]. As shown in Fig. 1, the energy level of the acceptor (NBD fluorephore) in excited state was estimated to be −11.34 eV. Obviously, the highest occupied molecule orbital (HOMO) energy level (−8.92 eV) of donor 1 (amino group in red box) was high enough to induce the PET, whereas the energy level (−11.76 eV) of donor 2 (α-KA group in red box) was too low to

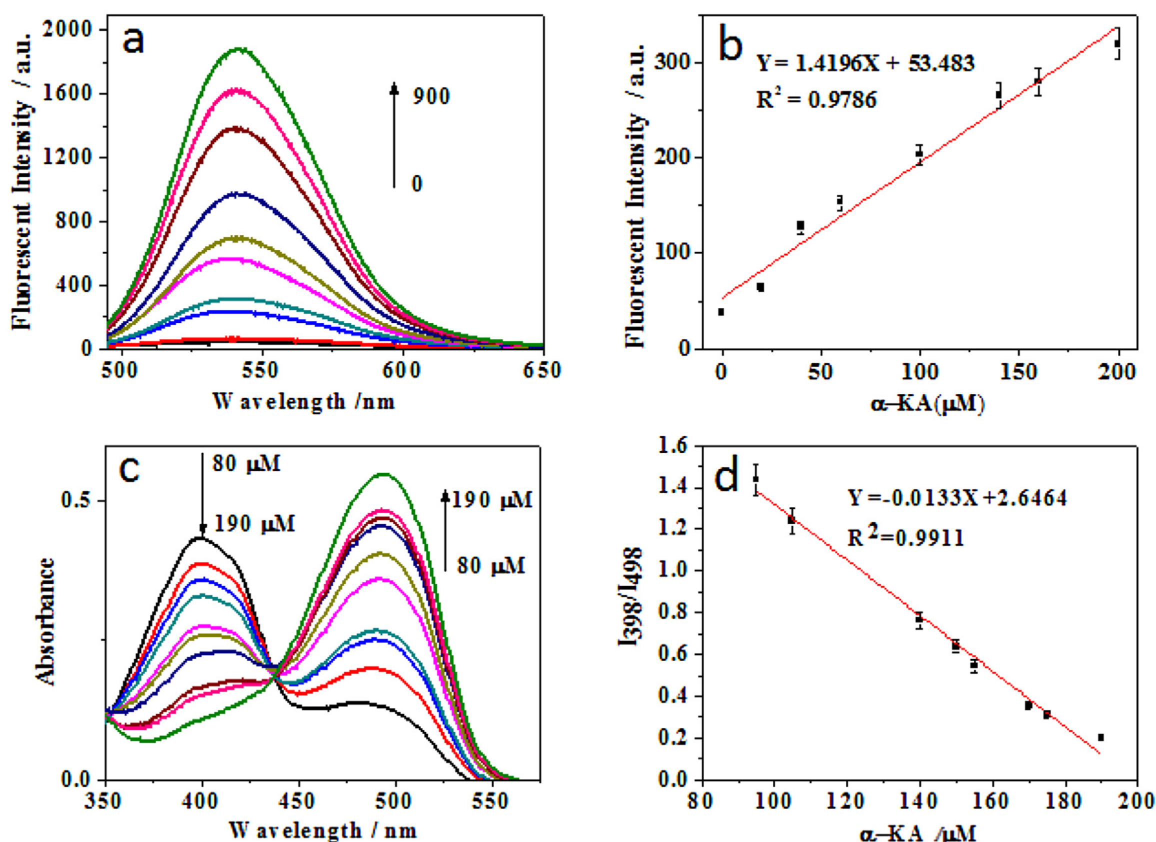

Fig. 2. (a) Fluorescence spectra and (b) the response linearity of HNBD probe (20 μM) upon the addition different α-KA concentrations from 0 to 200 μM (λ_{ex}: 475 nm, λ_{em}: 540 nm). (c) Ultraviolet spectra and (d) absorbance-ratio linearity of HNBD probe (20 μM) upon the addition of α-KA from 80 to 190 μM. Donor 1 and 2 are the groups in the red dashed frame. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

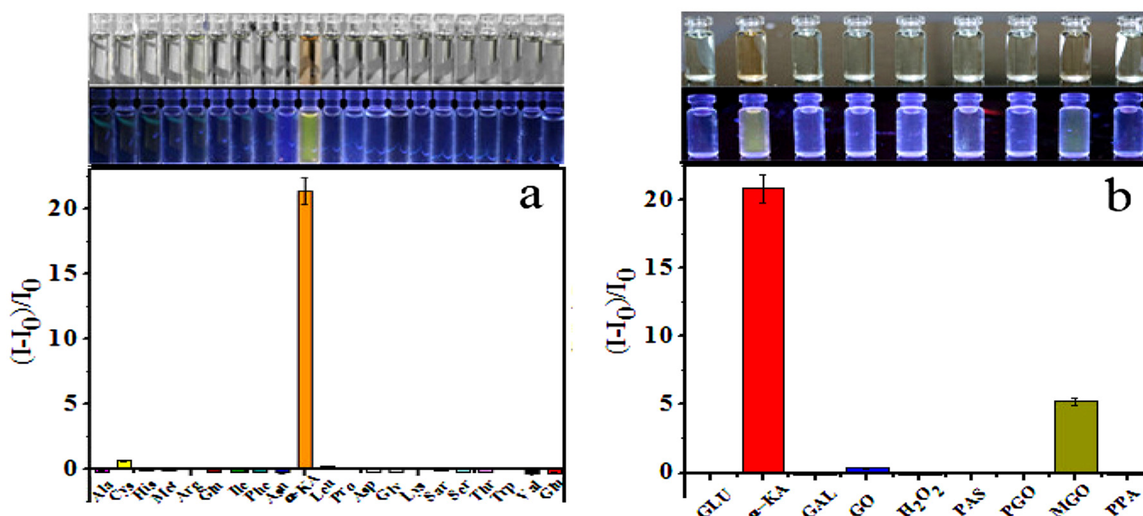

Fig. 3. Selective optical responses of HNBD probe (20 μM) in daylight (up) and ultraviolet light (down) to α-KA and other possible concomitants of (a) varying amino acids (100 μM) of Ala, Cys, His, Met, Arg, Gln, Ile, Phe, Asn, α-KA, Leu, Pro, Asp, Gly, Lys, Sar, Ser, Thr, Trp, Val, and Glu; and (b) other molecules of Glu (5.0 mM),α-KA (100 μM), Gal (200 μM), GO (200 μM), H_2O_2 (200 μM), PAS (200 μM), PGO (200 μM), MGO (200 μM), and PPA (200 μM).

cause an effective PET. On the basis of this PET mechanism, theoretically, the target α-KA would react with HNBD probe to inhibit the PET process, thereby turning on the fluorescence. As expected, a remarkable emission enhancement up to 60 fold (Fig. 2a) was obtained in the product of HNBD-α-KA (λ_{em}540 nm) comparing with HNBD probe, which confirmed that the rational design of PET mechanism led to the sensitive fluorescence off/on switching toward α-KA. Finally, we confirmed the PET mechanism with an experiment of pH-dependent fluorescence variation. Considering the basicity of diazanyl in HNBD, this experiment was designed to inhibit the PET process by neutralizing the amino group with hydrogen ion (ESI). What we need to demonstrate is the probe worked via the mechanism of whether PET or ICT. According to the literature[23], the ICT mechanism would present the blue-shift of

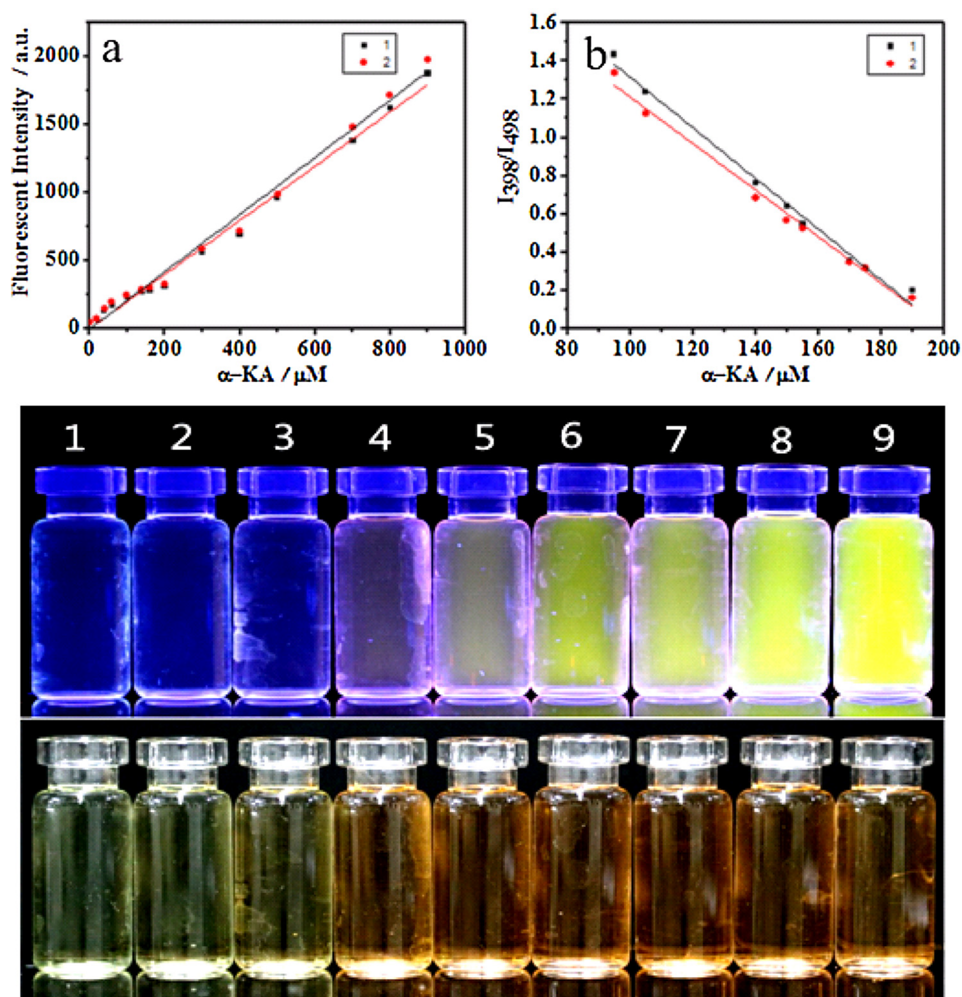

Fig. 4. Comparison of linear relationships of α-KA concentrations versus (a) fluorescence and (b) absorbance ratios between PBS (1 black) and serum samples (2 red); Photographs of HNBD probe (20 μM) in serum samples (1–9) spiked with various levels (0 to 800 μM) of α-KA in ultraviolet light and daylight (down). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

absorption/fluorescence emission if the amino nitrogen in diazanyl was protonated. Whereas, as can be seen, an obvious increase of fluorescent response appeared at the pH values below 3 (Fig. S3, ESI) and no shift of absorption/fluorescence was observed. Such fluorescence behaviour should be attributed to not the ICT but the PET mechanism. Overall, these findings demonstrated the proposed PET sensing mechanism of the developed HNBD toward α-KA.

Practically, in addition to the remarkable turn on of fluorescence, the product HNBD-α-KA also showed an unexpectedly large red shift of 100 nm in the absorption relative to HNBD (Fig. 2 c), exhibiting a the special optical mechanism of PET with red shift of absorbance for α-KA sensing. Comparing with HNBD, the reduced energy gap (0.27 eV) of HOMO-LUMO (lowest unoccupied molecular orbital) in HNBD-α-KA was probably responsible for this red shift. To our surprise, however, no red shift was observed in the fluorescent emission of probe HNBD, implying that a novel optical behaviour combining fluorescence turn on and absorption red shift was successfully designed for this α-KA probe. Maybe, it is the effective PET that quenched the original fluorescence, so that no shift of fluorescence was observed but an obvious turn on. Thus, a multifunctional application of HNBD could be expected in virtue of these distinctive properties, including off/on switch of fluorescence, naked eye monitoring, and absorption-ratiometric quantification, as demonstrated afterwards.

3.2. The selectivity of probe

Based on the strong turn-on of fluorescence, the selectivity of HNBD probe for α-KA over other possible concomitants was examined, including 20 types of amino acids, glucose (Glu), galactose (Gal), glyoxal (GO), hydrogen peroxide (H_2O_2), sodium pyruvate (PAS), phenylglyoxal (PGO), and methylglyoxal (MGO) (Fig. 3). Apparently, HNBD showed the high selectivity for reporting α-KA over co-existing amino acids. In addition, α-KA could be specifically detected in the media containing the excessive aldose, ketose, glyoxal, pyruvate acid, and phenylglyoxal (Fig. 3 b). Though 200 μM of Methylglyoxal displayed a weak response, it would cause no significant interference at its biological levels (generally <2.0 μM). Therefore, the developed probe HNBD showed the satisfactory specificity to α-KA under the experimental conditions.

3.3. Condition optimization

It can be seen from Fig. S4 that the developed HNBD probe could present the satisfactory responses to α-KA over the pH range of 5.4–8.9. Temperature investigations showed the maximum response of HNBD to α-KA at the temperature of 37 °C, which is in good consistency to the biological condition of living body (Fig. S5). In addition, electrolyte investigations displayed the response

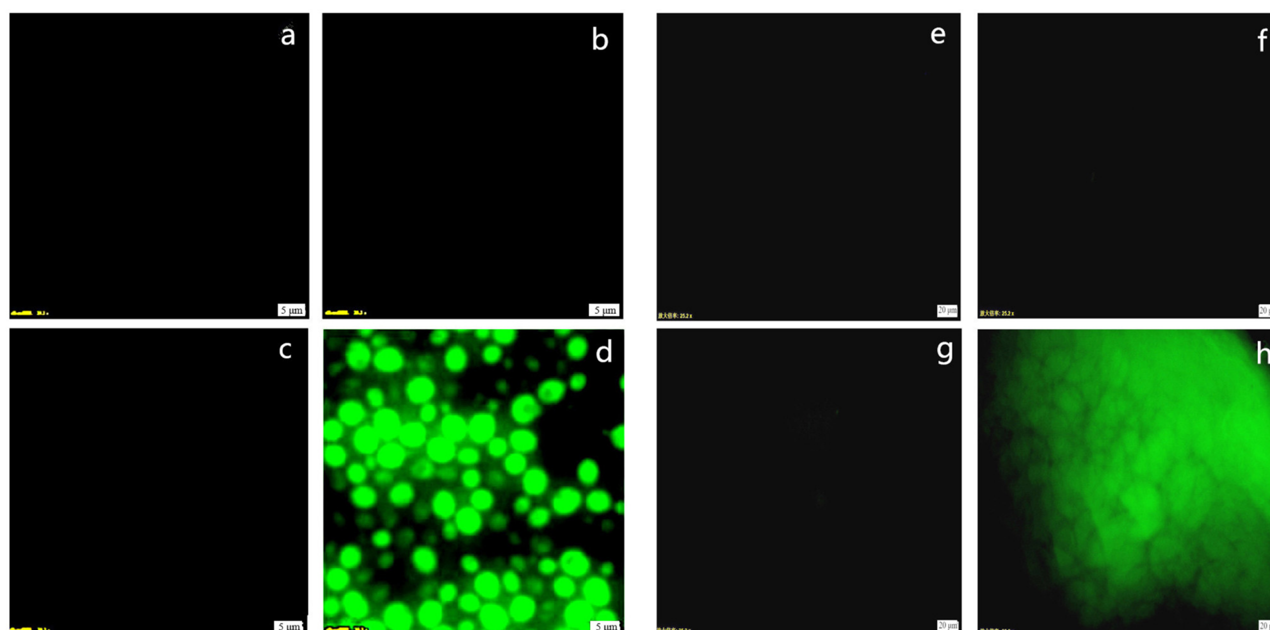

Fig. 5. Fluorescence images of yeast cells incubated separately in various media: (a) cell, (b) cell + α-KA (200 μM), (c) cell + HNBD probe (20 μM) and (d) cell + HNBD probe (20 μM) + α-KA (200 μM); fluorescence images of porcine tissue incubated separately in various media of (e) tissue, (f) tissue + α-KA (200 μM), (g) tissue + HNBD probe (20 μM), and (h) tissue + HNBD probe (20 μM) + α-KA (200 μM), where the media consists of yeast extract (1%), peptone (2%), and glucose (2%) in water sterilized under high pressure at 121 °C for 20 min.

of this probe to α-KA was not influenced by biological level of NaCl (100 mM) (Fig. S6). Therefore, the as-developed HNBD would be tailored as a promising candidate probe for the detection of α-KA in the biological fields.

3.4. Establishment of linear relationship

The dependence of fluorescence intensity on α-KA concentration was determined to find the linear relationship (Fig. 2). As can be seen from Fig. 2a and b, the wide linearity at the concentration of 2.0 to 900 μM was achieved and meanwhile the other acceptable linearity at the low concentration of 2.0 to 200 μM was obtained. The detection limit was estimated to be 0.9 μM, as calculated by the 3 σ rule. In addition, the large red shift (100 nm) in UV–vis absorption would also facilitate the absorbance-ratiometric analysis for targeting α-KA (Fig. 2c and d). A linear relationship describing the ratiometric changes in absorbance versus various α-KA concentrations could be realized in the linear range from 80 to 190 μM. Such a ratiometric detection format can present more accurate calibration than the conventional method. Also, considering the possible concentration level in biological materials [29], this ratiometric method can be specially suitable for probing α-KA in the biological system.

3.5. Applications to biological samples

3.5.1. Linear relationship and colorimetric analysis in serum

The unique optical properties of HNBD probe toward α-KA inspire us to develop its utility in the biosystem. First, HNBD was applied to quantify the human serum spiked by α-KA (Fig. S7). One can note that the absorption of HNBD probe in blank serum showed no significant change, so did its fluorescence, indicating that the designed probe survive well in the complicated matrices of serum. Upon addition of α-KA to the above serum media, the orange-red colour in daylight and green fluorescence in ultraviolet light were observed, allowing for the easy naked-eye monitoring of α-KA in serum samples (Fig. 4). Also, human serum solutions containing different levels of α-KA (2.0–900 μM) were prepared and incubated separately with 20 μM of HNBD. Results showed that human serum experiments presented the linearity correlating well with the linear relationship obtained from PBS solutions (Fig. 4). Thus, the accurate, efficient, and convenient detection/monitoring of α-KA in serum samples can be enabled with this probe.

3.5.2. Imaging of α-KA in yeast cell

The feasibility of fluorescence imaging of α-KA in living cells was explored (Fig. 5). Yeast cells were incubated with HNBD in presence and absence of α-KA, separately. First, without addition of α-KA, cells pre-treated by HNBD could exhibit almost no fluorescence emission (Fig. 5a–c), suggesting that HNBD probe was immune to various intracellular species under the culture conditions. In contrast, the addition of α-KA (10 equiv) to the above cells light up a green image under ultraviolet (Fig. 5d), indicating that HNBD probe was capable of imaging α-KA in living cells with good membrane permeability.

3.5.3. Imaging of α-KA in tissue

We further investigated the feasibility of HNBD probe for tissue imaging. Fresh pork muscle tissues were incubated with HNBD or α-KA, showing no significant fluorescence (Fig. 5e–g). After α-KA (10 equiv) was added into the HNBD-containing media, there appeared a remarkable increase in fluorescence intensity, as evidenced by the clear image (Fig. 5g). Therefore, HNBD probe could be used to image tissues with the satisfactory tissue penetration capacity.

4. Conclusions

In summary, we have initially developed a multifunctional optical probe for the accurate (ratiometric calibration), sensitive (60-fold enhancement) and convenient (naked-eye) detections as well as biological imaging of α-KA in serum, living cell, and tissue. This probe enjoys the controllable PET process and variable HOMO-LUMO energy gap, thus providing the remarkable turn-on

of fluorescence and a large red shift in absorption toward α-KA. The developed probe is essentially different to the routine α-KA probes featuring the ICT mechanism with the blue shift of absorption. Sensitive responses with a dramatic colour change can also allow for the naked eye monitoring of α-KA under day light or ultraviolet light. Moreover, the developed probe enables the quantification implemented simultaneously by the ratiometric absorption and the fluorescence intensity, so that an accurate self-calibration can be realized. In addition, the proposed fabrication protocol can find the extensive applications for the design of small-molecules optical probes for biological fields.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2016.10.044.

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Biographies

Qiang Fu is a student at the Qinghai normal university working for his master degree. His research interest includes design of organic probes for sensing biomakers of cancer.

Zhi Chen is a professor of Life and Geographical Sciences school of Qinghai normal University. His research interests are in the study of the animals in Qinghai-Tibet Plateau.

Xueying Ma is a senior student of Qufu normal university. She loves research about biosensor very much and is now working for the admission to be a master student in this university.

Professor Guang Chen has graduated with his Ph.D. from the Northwest Plateau Institute of Biology of Chinese Academy of Sciences, and now is a teacher and researcher in Qufu normal university. His research interests are focused on the design of organic probe, the exploring of sensing mechanism, the pretreatment techniques of derivatization. He has won the supports from National Natural Science Foundation of China, Natural Science Foundation of Shandong Province, and Open Funds of the Shandong Province Key Laboratory of Detection Technology for Tumor Markers to perform the extensive study of probing the small molecules, potential biomarkers and the corresponding techniques for application of sensors in biological samples.

Professor Yuxia Liu is a researcher in Qufu normal university, who does well in exploring the procedure and mechanism of chemical reaction with the aid of Gaussian program. She got the Ph.D. from Shandong university and now is studying the sensing mechanism as an important member of our research group.

Professor Ziping Cao used to be a researcher in Shanghai Institute of Organic Chemistry of Chinese Academy of Sciences and now becomes a teacher and researcher in Qufu normal university. He is good at synthesizing various organic compounds under the anhydrous, anaerobic conditions.

Professor Fei Qu is a young yet excellent researchers in Qufu normal university, who has achieved some experience for designing the sensors of silver-quantum-dot.

Professor Daoshan Yang is a teacher and researcher in Qufu normal university. He is good at developing environmentally friendly techniques for the synthesis of various organic compounds.

Professor Xianen Zhao is a teacher and researcher in Qufu normal university. His research interest is mainly in the components analysis of traditional Chinese herb. Also, the research on developing analytical method for living animals is his interest.

Professor Zhiwei Sun is a teacher and researcher in Qufu normal university. His research interest is about the metabolomics with the aid of HPLC coupled with MS. He is also good at developing the stable isotopic probes to establish the accurate, sensitive and rapid platform for metabolomics

Professor Guoliang Li is a teacher and researcher in Qufu normal university. His research interest is about the components analysis of traditional Chinese herb. Also, he is good at designing the carbon-quantum-dot for the study of biological procedures

Professor Shijuan Zhang is a teacher and researcher in Qufu normal university. His research interest is mainly in the components analysis of traditional Chinese herb.

Professor Fengli Qu is a teacher and researcher in Qufu normal university, who has graduated with her Ph.D. from the chemistry and chemical engineering school of Hunan university. She has got lots of achievements in designing biosensors, and has won the May Fourth Movement Literature Medal as a young lady.

Professor Rongmei Kong is a teacher and researcher in Qufu normal university, who has graduated from the chemistry and chemical engineering school of Hunan university and won the Ph.D. She is good at developing biosensors for the research of biological fields.

Professor Hua Wang was once a teacher and researcher in chemistry and chemical engineering school of Hunan university and now becomes a member of Qufu normal university. He has been working in Pacific Northwest National Laboratory as an assistant researcher and Singapore National Institute of Bioengineering and Nanotechnology as a senior research scientist. He has won the Taishan Scholar when he come to Qufu normal university. His research interests are in the fields of environmental and medical testing devices, nano-materials and surface treatment, organic synthesis and drug design, biomaterials and cell regulation in areas including basic research and applied technology research.

Professor Jinmao You, a scholar of CAS Hundred (No. 328) is now the directors of both the key laboratory of life-organic analysis and the Key Laboratory of Pharmaceutical Intermediates and Analysis of Natural Medicine, in Qufu Normal University. He has obtained some achievements in the fields of derivatization techniques used for the components analysis of various biological and environmental samples.